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NONENZYMATIC FIBRINOLYSIS DURING IMMOBILIZATION
STRESS IN ANIMALS

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16. Abstract The 30-minute immobilization stress was shown to raise significantly the non-enzymatic fibrinolytic activity of blood in rates. Combined with ACTH the effect is still greater. I. v. administration of 0.2 ml 0.01% solution of protaminsulphate prevented the non-enzymatic fibrinolysis induced by the stress. Administration of ACTH after protaminsulphate again raised the fibrinolysis. This suggests that ACTH stimulates the release of heparin.			
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THE ROLE OF ACTH AND GLUCOCORTICOIDS IN
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By B. A. Kudryashov,* F. B. Shapiro,* E. G. Lomovskaya,* and L. A. Lyapina*

In the adaptive humoral reactions to stress effects, one of the central /735**
places is undoubtedly occupied by processes directed both towards natural prevention of possible blood loss, and towards preservation of the fluid state of circulating blood, guarding the organism from possible thrombogenesis. It is known that these physiological processes that prevent thrombogenesis are associated primarily with the function of a very labile and reactive anticoagulative system. The studies conducted in this field have demonstrated that during excitation of the anticoagulative system in the blood complex compounds of heparin develop with blood proteins and certain hormones, that possess anticoagulative properties and the capacity to lyse unstable clusters of fibrin, i.e., to implement nonenzymatic fibrinolysis [4-6].

An important role in the activation of the function of the anticoagulative system during stress is played by the altered hormonal status of the organism that is due to intensified secretion of adrenaline, ACTH and glucocorticoids. Adrena-

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line, on the one hand, and ACTH-glucocorticoids, on the other hand, have a stimulating effect on the nonenzymatic fibrinolysis, intensifying the process of formation of complex heparin compounds. Any experimental effect during which the discharge of ACTH and glucocorticoids cannot be implemented completely under stress conditions or the action of adrenaline be realized (blockade of ACTH secretion, adrenalectomy, blockade of α -adrenoreception), naturally results in inhibition of /736 the nonenzymatic fibrinolysis, or in other words, in a decrease in the functional activity of the anticoagulative system [13, 14]. Here one should bear in mind, that the stimulating effect of ACTH-glucocorticoids and adrenaline on the process of complex heparin compound formation has an independent nature and is not summed up.

Thus, in the organism there exist two parallel paths for guarantee of intensified nonenzymatic fibrinolysis during stress effects on the organism.

The stimulating effect of adrenaline on the process of formation of heparin complexes is mediated through developing thrombinogenesis [3, 15, 24], that naturally stimulates the anticoagulative system and thereby even activation of the nonenzymatic fibrinolysis. Here the thrombogenic effect of adrenaline is implemented through the autonomic nervous system and α -adrenergic reception [8, 9].

It is still difficult to say anything definite about the mechanism of action of ACTH-glucocorticoids. The possibility is not excluded that here stimulation of blood coagulation and subsequent activation of the function of the anticoagulative system also occur. But it is quite possible that some other mechanisms lie at the basis of the stimulating effect of the ACTH-glucocorticoids. In this respect the question is important of whether ACTH-glucocorticoids induce intensification of the nonenzymatic fibrinolysis, by stimulating the discharge of heparin, since it is known that during activation of the anticoagulative system intensifi-

cation of formation of complex heparin compounds is preceded by discharge of heparin and activators of plasminogen into the blood. In order to answer this question, we decided to clarify whether discharge of heparin occurs under the influence of ACTH-glucocorticoids during a stress effect, and whether the non-enzymatic fibrinolysis is correspondingly intensified, if heparin is preliminarily bonded by the administration of protamine sulfate in animals in vivo.

TECHNIQUE

Work was conducted on mongrel male rats weighing 170-200 g. The stress state was induced by 30-minute immobilization (tying to a table). Blood was taken from the v. jugularis with sodium citrate in a ratio of 9:1. The total fibrinolytic activity of the blood was determined according to the method of Astrup and Mullertz [21], the nonenzymatic fibrinolysis implemented by the complex heparin compounds—according to the method of B. A. Kudryashov and L. A. Lyapina with epsilon-amino-caproic acid (EACA) that inhibits the effect of plasmin [7]. Protamine sulfate (firm SPOFA) in a physiological solution (0.01%) in different doses was administered intravenously; five units of ACTH (S. M. Kirov Meat Combine) and 1 and 2 mg/100 g of hydrocortisone (firm Gedeon Rikhter) in a physiological solution intra-abdominally within 15 minutes after administration of protamine sulfate, when a complex protamine sulfate-heparin had already been formed. The volume of the administered substances was 1 ml; in all cases an equal volume of physiological solution was administered intravenously or intra-abdominally for the control. In a determination of the fibrinolytic activity on the unstabilized platelets of fibrin by factor 13, prepared according to the method of B. A. Kudryashov, L. A. Lyapina, and I. P. Baskova [12], 0.05 ml of plasma with a physiological solution or EACA were applied, and incubated for 2 hours at 37°. The magnitude of the fibrinolytic

activity was judged according to the size of the lysis zone on the fibrin platelets per 1 mm².

RESULTS OF STUDY AND THEIR DISCUSSION

To answer the question of whether release of heparin occurs under the influence of ACTH-glucocorticoids it was decided, as already indicated above to bond heparin with the help of protamine sulfate that forms with it, as is known, a stable complex inactive in a lytic sense, and thereby under stress conditions to block the possible formation of other complex heparin compounds that possess fibrinolytic activity, i.e., to block nonenzymatic fibrinolysis. Complete blockade of the lytic activity of the complex heparin compounds with the help of protamine sulfate in vitro is a known fact [10]. In that case, if after bonding of heparin by protamine sulfate in vivo nonenzymatic fibrinolysis again would begin to be /737 implemented under the influence of ACTH-glucocorticoids, there would be all grounds to consider that they induced additional release of heparin.

First of all it was necessary to determine the threshold dose of protamine sulfate, for with the administration of a surplus of protamine sulfate, evidently, the newly released heparin would also be bonded. Table 1 presents results of the determination of the nonenzymatic fibrinolytic activity in rats who received an intravenous dose of a varying quantity of protamine sulfate and were exposed to immobilization stress.

As one can see from these data, the threshold dose of protamine sulfate for bonding heparin in vivo under our experimental conditions is 0.2 ml of a 0.01% solution. With the administration of this quantity of protamine sulfate the nonenzymatic fibrinolytic activity during stress is practically not manifest (group 5);

its absolute amount is only 2.0 mm^2 , while the percentage in the total fibrinolytic activity of blood is 4.3%. Moreover, in the control animals who received a physiological solution these amounts respectively equalled 37.1 mm^2 and 46.7% (group 1). The administration of 0.1 ml of protamine sulfate was insufficient for bonding heparin, and nonenzymatic fibrinolysis during stress remains the same as in the control animals (group 6). The administration of 0.3–0.5 ml of protamine sulfate completely blocks nonenzymatic fibrinolysis, but evidently if these doses are used we will a fortiori introduce a surplus of protamine sulfate, on whose background the new release of heparin will be difficult to catch.

TABLE 1. NONENZYMATIC FIBRINOLYTIC ACTIVITY DURING IMMOBILIZATION STRESS AFTER ADMINISTRATION OF PROTAMINE SULFATE

Group of Animals	No. of Animals	Zone of Lysis (mm^2) (A)	Zone of Lysis + EACA (mm^2) (B)	$\frac{B \times 100}{A}$
Physiological solution	24	77.5 ± 4.3	37.1 ± 1.9	46.7 ± 2.0
0.5 ml of protamine sulfate	10	38.1 ± 5.3	0	0
0.4 ml "	6	35.3 ± 3.2	0	0
0.3 ml "	6	34.5 ± 4.2	0	0
0.2 ml "	9	43.9 ± 2.6	2.0 ± 1.0	4.3 ± 0.5
0.1 ml "	7	75.3 ± 3.1	39.3 ± 4.4	51.3 ± 2.3

Table 2 presents data on the magnitude of nonenzymatic fibrinolytic activity in animals who received ACTH after heparin was bonded in them by the administration of a different quantity of protamine sulfate.

TABLE 2. NONENZYMATIC FIBRINOLYTIC ACTIVITY DURING IMMOBILIZATION STRESS AFTER ADMINISTRATION OF PROTAMINE SULFATE AND ACTH

Group of Animals	No. of Animals	Zone of Lysis (mm^2) (A)	Zone of Lysis + EACA (mm^2) (B)	$\frac{B \times 100}{A}$
ACTH	36	136.7 ± 4.2	80.3 ± 2.3	60.0 ± 0.4
0.4 ml protamine sulfate + ACTH	9	46.4 ± 4.3	0	0
0.3 ml "	8	42.1 ± 7.2	13.2 ± 5.0	31.3 ± 7.7
0.2 ml "	22	77.7 ± 3.7	46.0 ± 4.1	58.0 ± 2.4

As we see, the administration of ACTH after a threshold dose of protamine sulfate (0.2 ml) resulted in the fact that formation of complex heparin compounds began again, and nonenzymatic fibrinolysis again reaches the level characteristic for combined immobilization stress and ACTH (groups 1 and 4), when the percentage /738 of nonenzymatic fibrinolytic activity in the total fibrinolytic activity of the blood is ~60%. ACTH administration on a background of large doses of protamine sulfate (0.3-0.4 ml) yielded, as should be expected, an effect that is inversely proportional to the surplus protamine sulfate introduced into the organism (groups 2 and 3), that again bond the released heparin. Thus, there are all the grounds to believe that ACTH stimulates heparin release and at the same time creates the possibility for a significant intensification in nonenzymatic fibrinolysis.

Naturally the question arises of whether this effect of ACTH is mediated through glucocorticoids; if it is mediated through activation of the adrenal cortex, then the release of heparin evidently can also be induced by the administration of a glucocorticoid hormone. To explain this, after bonding heparin with protamine sulfate we administered to the rats under immobilization stress not ACTH, but that quantity of hydrocortisone (1 mg/100 g) that, as we previously established [11], normalizes the formation of complex heparin compounds in adrenalectomized rats in a stress state. The findings are presented in Table 3 and Figure 1. It was found that administration of 1 mg/100 g of hydrocortisone in a background of protamine sulfate did not result in a new release of heparin, and nonenzymatic fibrinolytic activity practically remained on the same level as with administration of only protamine sulfate (groups 1 and 2). The pattern was not altered by the administration of a double quantity of hydrocortisone (2 mg/100 g); no release of heparin /739 followed and nonenzymatic fibrinolytic activity was not increased (group 3). The

TABLE 3. NONENZYMATIC FIBRINOLYTIC ACTIVITY DURING IMMOBILIZATION STRESS AFTER ADMINISTRATION OF PROTAMINE SULFATE AND HYDROCORTISONE

Group of Animals	No. of Animals	Zone of Lysis (mm ²) (A)	Zone of Lysis + EACA (mm ²) (B)	$\frac{B \times 100}{A}$
0.2 ml protamine sulfate	9	43.9 ± 2.6	2.0 ± 1.0	4.3 ± 0.5
0.2 ml protamine sulfate + 1 mg/100 g hydrocortisone	5	68.4 ± 4.7	8.4 ± 6.1	12.3 ± 6.7
0.2 ml protamine sulfate + 2 mg/100 g hydrocortisone	9	42.5 ± 3.0	2.7 ± 1.8	5.5 ± 4.0

absence of an additional release of heparin under the influence of hydrocortisone is indicated by the fact that its administration in contrast to the administration of ACTH does not result, under conditions of stress effect, in a further increase in nonenzymatic fibrinolysis even in animals that received protamine sulfate. If, as is apparent from the data of Table 4 (groups 1 and 2) and Figure 1, the administration of ACTH increases, during immobilization, the absolute amount of nonenzymatic fibrinolytic activity 2.5-fold (from 32.9 to 80.3 mm²), and its percentage in the total fibrinolytic activity of blood 1.5-fold (from 40 to 60%), then the administration of hydrocortisone (1 and 2 mg/100 g) does not yield an effect, and nonenzymatic fibrinolytic activity remains the same as in the animals exposed only to immobilization (groups 3 and 4).

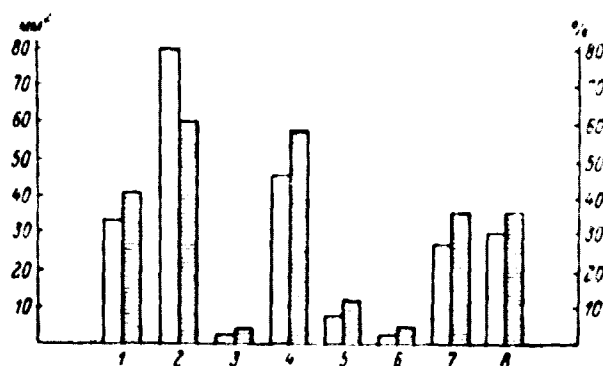


Figure 1. Nonenzymatic Fibrinolytic Activity in Intact Rats During Immobilization Stress After Administration of Protamine Sulfate, ACTH and Hydrocortisone
[Key on next page]

Key:

Light columns—absolute amount, in mm^2 ; shaded columns—percentage of total fibrinolytic activity

- | | |
|-----------------------------------|---|
| 1. Physiological solution | 5. 0.2 ml protamine sulfate + 1 mg/100 g hydrocortisone |
| 2. ACTH | 6. Same + 2 mg/100 g hydrocortisone |
| 3. 0.2 ml protamine sulfate | 7. 1 mg/100 g hydrocortisone |
| 4. 0.2 ml protamine sulfate +ACTH | 8. 2 mg/100 g hydrocortisone |

TABLE 4. NONENZYMATIC FIBRINOLYTIC ACTIVITY DURING IMMOBILIZATION STRESS AFTER ADMINISTRATION OF ACTH AND HYDROCORTISONE

Group of Animals	No. of Animals	Zone of Lysis (mm^2) (A)	Zone of Lysis (mm^2) +EACA (B)	$\frac{B \times 100}{A}$
Physiological solution	20	76.0 ± 6.2	32.9 ± 4.0	40.8 ± 4.0
ACTH	36	136.7 ± 4.2	80.3 ± 2.3	60.0 ± 0.4
Hydrocortisone 1 mg/100 g	14	76.1 ± 3.0	26.9 ± 1.5	35.6 ± 1.6
Hydrocortisone 2 mg/100 g	15	83.0 ± 4.5	30.4 ± 2.2	36.4 ± 1.6

Since hydrocortisone did not induce release of heparin, it should be considered that the stimulating effect of ACTH on this release is not mediated through activation of corticosteroid secretion.

What then is the role of corticosteroids? It is known that ACTH in the absence of glucocorticoids does not increase nonenzymatic fibrinolytic activity in adrenalectomized rats in a stress situation, while hydrocortisone normalizes in them the reaction of the anticoagulative system to the stress effect, and increases the nonenzymatic fibrinolysis to a level characteristic for intact animals [11].

Evidently here the permissive effect of glucocorticoids is displayed, which as is known, is also expressed in the fact that a certain concentration of glucocorticoids in the organism is necessary for implementation of a number of physiological and biochemical reactions. This is indicated by the results of the experiments we conducted with bonding heparin by protamine sulfate in adrenalectomized rats within 48 h after removal of the adrenal glands, when an acute deficit of

corticosteroid hormones occurs in them [20]. Corresponding data are presented /740
in Table 5 and Figure 2.

TABLE 5. NONENZYMATIC FIBRINOLYTIC ACTIVITY DURING IMMOBILIZATION STRESS IN ADRENALECTOMIZED ANIMALS AFTER ADMINISTRATION OF PROTAMINE SULFATE, ACTH AND HYDROCORTISONE

Group of Animals	No. of Animals	Zone of Lysis (mm ²) (A)	Zone of Lysis +EACA (mm ²) (B)	$\frac{B \times 100}{A}$
Physiological solution	10	49.7 ± 2.4	12.8 ± 1.7	25.7 ± 3.4
0.2 ml protamine sulfate	8	21.1 ± 2.7	0	0
0.2 ml protamine sulfate +ACTH	14	33.5 ± 2.0	5.6 ± 1.3	16.0 ± 3.6
0.2 ml protamine sulfate + 1 mg/100 g hydrocortisone	13	43.9 ± 2.3	4.6 ± 1.5	10.1 ± 3.3
0.2 ml protamine sulfate +ACTH + 1 mg/100 g hydrocortisone	8	50.9 ± 2.5	12.2 ± 1.5	23.4 ± 2.5

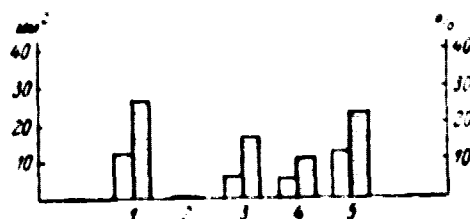


Figure 2. Nonenzymatic Fibrinolytic Activity in Adrenalectomized Rats During Immobilization Stress After Administration of Protamine Sulfate, ACTH and Hydrocortisone

Key:

1. Physiological solution
2. 0.2 ml protamine sulfate
3. 0.2 ml protamine sulfate +ACTH
4. 0.2 ml protamine sulfate + 1 mg/100 g hydrocortisone
5. 0.2 ml protamine sulfate + ACTH + 1 mg/100 g hydrocortisone

Remaining designations the same as in Figure 1.

As one can see from the data given in Table 5, protamine sulfate bonded heparin in the adrenalectomized animals and completely blocked nonenzymatic fibrinolysis (group 2). The administration of ACTH on a background of protamine sulfate (group 3),

having induced a conditional release of heparin, stimulated nonenzymatic fibrinolysis, but it did not reach the level characteristic for adrenalectomized animals exposed to immobilization stress (group 1); it was 40% lower. An even lower effect was yielded by the administration of glucocorticoids on the background of protamine sulfate (group 4): in this case the nonenzymatic fibrinolytic activity was 2.5-fold lower than in the adrenalectomized animals that did not receive protamine sulfate, and its percentage in the total fibrinolytic activity of the blood was only 10.1%, i.e., was the same as in the intact rats who received hydrocortisone after bonding of heparin by protamine sulfate (compare Table 3 groups 2 and 3). We see a completely different picture in the adrenalectomized animals who received after protamine sulfate, ACTH and hydrocortisone (group 5): in them the nonenzymatic fibrinolytic activity, both according to the absolute amount and the percentage in the total fibrinolytic activity of the blood, again reached that level that is characteristic for adrenalectomized animals exposed to the stress effect without bonding of heparin by protamine sulfate.

Thus, we see that for formation of complex heparin compounds that implement nonenzymatic fibrinolysis, a small role is played by the presence of a sufficient titer of heparin in the blood, which evidently is guaranteed in a stress situation, in particular by ACTH. A certain (physiological) concentration of glucocorticoid is also necessary, without whose permissive activity this complex-formation cannot be successfully realized.

In light of the permissive effect of glucocorticoids it becomes understandable how the presence of a high level of heparin in the blood is combined with reduced process of complex heparin compound formation in adrenalectomized rats in certain periods after removal of the adrenal glands. The high heparin content in blood after

adrenalectomy is a known fact [16, 19, 27]. One can justifiably hypothesize that this high heparin content to a certain measure is determined by the ACTH secretion increasing after removal of the adrenal glands [22, 25, 26]. Nonenzymatic fibrinolysis in the adrenalectomized animals is retarded because for its implementation, as we have seen, the presence of glucocorticoids is necessary.

In conclusion one should state that the literature on the effect of ACTH on heparin content in the blood is fairly contradictory. One can name a number of experimental and clinical works that speak of an increase in the heparin titer with administration, especially multiple, of ACTH [1, 17, 18, 23]. On the other hand, there are known works that indicate the decrease in heparin content under the influence of ACTH [2]. It is possible that the reason for these disagreements is hidden to a certain measure in the fact that ACTH, stimulating the release of heparin, also intensifies the formation of its complex compounds, and consequently, in each case, /740 it is evidently necessary to take into consideration the inverse relationship between the heparin level in the blood and the intensity of formation of its complex compounds.

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